THE EFFECTS OF LEUCOGENENOL ON BENZENE INDUCED BONE MARROW SUPPRESSION IN RABBITS

An abstract of a Thesis by
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April 1976
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The problem. These experiments were to determine the extent of leucocytosis and antibody production, stimulated by leucogenenol, in an animal whose white blood cells have been reduced by the action of benzene.

Procedure. The white blood cell counts of rabbits were depressed to 1500 ± 500 cells per cubic millimeter by the action of benzene. At this time the animal, depending upon the grouping, was either given an injection of leucogenenol (2.0 or 0.2 µg) or allowed to recover on its own. Five groups of rabbits were maintained: Group 1, Control animals; Group 2, Benzene only; Group 3, Leucogenenol only; Group 4, Benzene and 0.2 µg of Leucogenenol; Group 5, Benzene and 2.0 µg of Leucogenenol.

Findings. The variations of the rabbits blood profile did not provide significant results. The data seemed to indicate possibilities rather than firm conclusions. The differences between the control group and the experimental group were only slight and not clear or directional.

Conclusion. The findings, although slight, indicate that leucogenenol does stimulate leucocytosis and antibody formation.

Recommendations. With the use of an agent such as benzene, the effects of leucogenenol can be easily observed. The rabbit as the experimental animal should be changed to one more suitable.

THE EFFECTS OF LEUCOGENENOL ON BENZENE INDUCED BONE MARROW SUPPRESSION IN RABBITS

A Thesis

Presented to

The School of Graduate Studies

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

Joseph Francis Talluto

April 1976

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INTRODUCTION AND REVIEW OF THE LITERATURE

Leucogenenol is a chemical agent which has been shown to initiate leucocytosis and numerous studies have been made to determine the relationship between the substance and hemopoietic activity. By inducing anemia in experimental animals, the extent of leucocytosis through leucogenenol stimulation can be studied.

Aplastic anemia is a disease in which the bone marrow fails to produce an adequate number of blood cells. There may be a lack of all types of blood cells (pancytopenia), or a deficiency of one or more cell types. The disease may be congenital as in Fanconi Syndrome, or it may be acquired by humans and other animals through exposure to certain chemicals such as benzene, or to ionizing radiation (Moore, 1971). Benzene is the simplest aromatic hydrocarbon and widely used in industry because of its solvent properties.

There are two forms of benzene intoxication, an acute form in which an animal receives a massive dose and a chronic form in which an animal receives small casual doses of benzene over a period of time. The chronic form of benzene poisoning produces bone marrow aplasia where varying numbers of the hemopoietic cells are involved. Forni et al. (1971) have also found a relationship between chronic benzene poisoning and chromosomal aberrations.

Although chemical compounds containing the benzene ring are numerous, benzene is the major myelotoxic agent and

not other hydrocarbons (Gerarde, 1960).

Benzene has an affinity for the fatty tissues rather than other body tissues. Elimination of benzene from the tissues is rapid at first and then slows because of the large amount of benzene held in fatty tissues and the poor blood supply to these deposits. Rabbits excrete 35% of the absorbed benzene as metabolites in the urine, the poison being completely removed in 2 to 5½ days with approximately 80 to 90% efficiency. The metabolites consist mainly of conjugated phenols; benzene, phenol, either catechol or hydroquinone, 1,2,4, trihydroxybenzene, and finally muconic acid (Stewart and Stolman, 1960).

In experimental animals the toxicities of benzene, toluene, and the three xylenes are similar whether they are given by injection or by inhalation. The low vapor pressure of xylene reduces the inhalation hazard. In large amounts these compounds of benzene, depress the central nervous system, repeated exposure to small amounts of benzene--depresses the bone marrow. In fatalities from chronic exposure to benzene, the findings include severe bone marrow aplasia, anemia, necrosis or fatty degeneration of the heart, liver, and adrenals (Dreisbach, 1969).

Experiments with chronic benzene poisoning in animals were first conducted by C. G. Santesson in 1897 (cited by Hamilton, 1931). In 1910-1911, L. Selling did the first

major study with benzene induced leucopenia. His technique of subcutaneously injecting equal volumes of benzene and olive oil, has been widely used by others. Selling (1910) found that injections of benzene and olive oil could severely lower the white cell counts of peripheral blood. The average loss of red blood cells was 16%, while the loss of white cells averaged 95% over an eight day period. He noted, as did others, that there was occasionally an increase in the white cell counts at the onset of the injections, with a rapid decline upon further injections (Hamilton, 1931). Selling (1910) stated that benzene either destroyed the peripheral leucocytes or else prevented new leucocytes from forming. However, he argued that 75% of leucocytes would not naturally die within 3 days, thus he favored the toxic effect of benzene. He found that the polymorpho-nuclear neutrophils were affected most when comparing injury in the bone marrows with that in the lymphoid tissue. Small lymphocytes are found in the greatest number and have the most resistance, and apparently increase after all other leucocytes have been destroyed.

Selling's studies were the basis for the deliberate utilization of benzene to reduce the white cell counts in leukemic patients. F. Billings (1913) supported the therapeutic use of benzene in the treatment for various forms of leukemia. Billings successfully treated a woman with leukemia by using benzene, and reduced her white cell

count from 150,000 to about 8,000. At home the woman continued treatment without authorization, and later returned to the hospital very anemic and with a white cell count of 1,400. She died several months later.

In their attempts to repeat Selling's work, Pappenheim and Neumann (Hamilton, 1931) found leucocytes abundant in the dilated capillaries of the liver, lungs, spleen, and kidneys, whereas they had almost disappeared from the peripheral circulation. Most authors report a general depletion of white cells in all parts of the organism.

Several experiments have been conducted to determine the effects of benzene on antigens and antibodies. Rusk and Schiff (Hamilton, 1931) working separately in 1914 tested the effect of benzene on antibody formation by injecting benzene into rabbits before or at the time of injection of the antigen. They found a decided reduction in the formation of lysin for sheep red blood cells and for precipitin. Simonds and Jones (1915) found that the ability to produce hemolysins for dog's blood is much reduced by benzene, as is the production of agglutinin and opsonins. Hektoen (1916) studied the action of benzene on the production of antibodies and the activity of leucocytes. He concluded that benzene may lower the anti-infection powers of the body in at least three ways: 1) by reduction of antibodies, 2) by reduction in the number of leucocytes, and

3) by a reduction of the phagocytic action of the leucocytes.

Camp and Baumgartner (1915) studied the effect of benzene on the course of the inflammatory process in rabbits induced by croton oil. Croton oil normally causes a marked inflammatory reaction in twenty-four to forty-eight hours. In the animals treated with benzene, no gross changes were found except pronounced edema, masses of bacteria, and the absence of leucocytes.

Hurwitz and Drinker (1915) studied the effects of benzene on the various factors of coagulation, primarily prothrombin and fibrinogen. They found, as did other authors, that the red blood corpuscles and the platelets were much less affected by benzene than were the leucocytes. They also found that benzene reduced the amount of circulating prothrombin and that this substance is dependent, in part for its production upon bone marrow activity.

Leucogenenol was first isolated by Rice (1966) from Penicillium gilmanii and later by Rice and Shaikh (1970) from normal bovine and human liver. Chemical analysis (Rice, 1971) indicated that the compound is an enol of the formula $C_{18}H_{25}NO_8$ and is 2-(1,2,dihydroxy-3-methyl-5-oxycyclohexyl)-3,11-(hydroxymethyl)-9 methyl-1-oxa-5-azaspiro 5,5 undeca-2,4 dien-7-one. The molecule is of importance because of its proposed ability to induce leucocytosis without a febrile response when injected into

rabbits (Rice, 1966). F. A. H. Rice (1966), writing in the same article, stated he found no correlation between the amount of leucogenenol injected and the degree of leucocytosis. At 0.02-0.2 micrograms/kilo., all animals (rabbits) showed a 2 to 3 fold increase in white cells. His observations indicated that injection of a microgram or more of leucogenenol showed a marked transient leucocytosis within the first hour and that this was followed (in mice) by leucopenia, but he does not state whether or not the leucopenia returned to a normal or lower leucocyte level.

In a later study, Rice (1968), found that the degree of leucocytosis induced by injection of leucogenenol varied considerably with the individual rabbit, as well as with the amount of leucogenenol injected. Rice found so much variation that an attempt to average the results would give erroneous conclusions. Leucogenenol seemed to cause an increase in the number of neutrophils, but the animals showed little if any change in the peripheral concentration of lymphocytes. Rice (1968) stated that since the effects of leucogenenol are prolonged rather than immediate, it is unlikely that it causes a release of cells from lungs, liver, or spleen.

Further studies by Rice and Darden, on the effects of leucogenenol on the blood cells of bone marrow in mice, indicate an increase in the concentration of the granulocytes as compared to the lymphocytes. Leucogenenol causes an

increase in the number of myeloblasts in the bone marrow, but little or no increase in basophils, megakaryocytes, eosinophils, or nucleated erythroid cells (Rice and Darden, 1968).

In studies using irradiated mice, Rice, Lepick, and Darden (1968) suggested that leucogenenol stimulated the maturation and/or cellular division of both the myeloid and lymphoid cells. The irradiated mice recovered more quickly with the use of leucogenenol than those not treated. They suggest that the results may be due to the possibility that leucogenenol acted as a coenzyme or portion of an enzyme in the stimulation of the maturation of blood cells.

Leucogenenol, isolated from bovine and human liver tissue, was found to be the same as the compound originally isolated from cultures of P. gilmanii (Rice and Shaikh, 1970). Injection of this compound into animals caused an increase in the number of peripheral neutrophils within 4-12 hours, and an increase in peripheral lymphocytes within 12-24 hours. After 24 hours there was a 2 to 3 fold increase in the relative number of myeloblasts in the bone marrow. This supported the earlier work of Rice, Lepick, and Darden (1968).

A. Smith (1929) treated an individual with marked leukopenia with several blood transfusions which proved ineffective. Smith then placed the patient on a liver diet, one-half pound daily for two months. The patient was

discharged with a leucocyte level of 5,800. The author does not elude to why a liver diet was chosen or how the liver was prepared.

If leucogenenol has an effect on individual cells, there should be an increase in the respiratory quotient and replication rate of cells grown in tissue culture. Cells used for such studies were murine leukemic cells of mice and human normal hymphoblastoid cells. It was found that leucogenenol does affect both the respiratory quotient and replication rate, probably by playing a role in the growth and regulation of the number of blood cells in the body (Rice, Blum, and Rene, 1970).

The study of leucogenenol for its effects on the production of antibody titers was done by Rice, Lepick, and Hepner (1970) who injected leucogenenol into irradiated mice. In the irradiated mice, antibody titers to sheep red blood cells showed significantly higher titers than those not given leucogenenol. Mean titers were also higher in the leucogenenol injected mice. However, normal mice showed no significant change either in the latent period or peak titer after being injected with leucogenenol. The effect of the low peak titers may be due to the effect of leucogenenol on the overall protein synthesis required for the recovery of systems unrelated to hemolysin formation, which in turn limited the production of antibodies. This study did not consider the possibility that the increase was due

to an increase in the number of circulating leucocytes which may produce more antibodies than normal.

Rice, McCurdy, and Aziz (1971) conducted a study to find if leucogenenol acted on one or more types of myeloid and erythroid tissues, and one or more lymphoid cells. The results of the experiment suggests that leucogenenol increases the rate at which myeloid cells are transformed. There seems to be little or no effect on erythroid cells.

In further studies to determine the effects of leucogenenol on the release of neutrophils and lymphocytes into the blood stream, Rice, Connolly, Aziz, and McCurdy (1971) used tritiated thymidine as a neutrophil label and lymphocyte label. The results indicate that leucogenenol causes a release of a large enough concentration of labeled neutrophils to be considered significant. The study also indicated that leucogenenol stimulated the rate of lymphocyte formation from the precursor cells.

To study the possibility of leucogenenol being an antibody stimulator, Rice et al. (1972) studied the effects of leucogenenol on the production of hemolysin in splenectomized rats. Their results indicate that leucogenenol does stimulate cells potentially capable of antibody synthesis. Leucogenenol seemed to have little effect in raising the hemolysis titers in unsplenectomized or normal rats, which may suggest that leucogenenol increases the rate of transformation of antibody-producing cells.

The effect of leucogenenol on the maturation of antibody producing cells was studied by Rice et al. (1972) who noted that it had been reported that splenectomy abolishes the early Ig M response but not the Ig G response. Hence, it was of interest to study the effect of leucogenenol on the formation of Ig M and Ig G hemolysin in normal and splenectomized rats. Their results suggests that leucogenenol affects neither the concentration nor the distribution of Ig M or Ig G hemolysin. Injections of leucogenenol into normal rats showed no increase in the hemolysin titers. It was found, however, that leucogenenol does increase the hemolysin Ig M titers in splenectomized rats suggesting that leucogenenol acts by increasing the rate of transformation of immuno-incompetent cells, possibly lymphoid, into antigen-reactive lymphocytes.

The purpose of this investigation was to study the function of leucogenenol in its ability to re-establish normal neutrophil and lymphocyte levels in rabbits rendered leucopenic by the toxic effects of benzene. In addition, studies were made to determine if leucogenenol can stimulate antibody production in a system almost entirely devoid of its normal antibody mechanism.

S. Schermer's book, The Blood Morphology of Laboratory Animals (1967), contains information about the blood characteristics of the rabbit. The normal blood cell counts of the rabbit include: 5 million erythrocytes, and 5,000 to

12,000 leucocytes per cubic millimeter with the neutrophils ranging from 8 to 50%, and the lymphocytes ranging from 20 to 90%.

METHODS AND MATERIALS

Young white female rabbits weighing between 1.5 and 3.1 kilograms were obtained locally. The animals were housed in groups of two or three to a standard, stainless steel cage. Cages were cleaned with a detergent solution and disinfected before use. The rabbits were fed a standard diet of Purina Rabbit Chow, and given water ad libitum. The diet was supplemented from time to time with celery, carrots, and lettuce.

The benzene (Fisher Scientific, analytical grade) used for this study was filtered through a Millipore filter and stored in a hermetically sealed acid-washed pyrex flask.

Commercial grade Pompeian olive oil was obtained locally and filtered through a Millipore filter requiring about four hours to complete. The filtered oil was mixed with the filtered benzene in a 1:1 ratio (Selling, 1910). In all experiments, benzene and olive oil were administered in 1 ml. doses/kilogram of body weight, given in divided doses.

Leucogenenol was isolated from lyophilized bovine liver (Nutritional Biochemical Corporation, Cleveland, Ohio), according to the procedure of Rice and Shaikh (1970).

Injections of 2.0 micrograms or 0.2 micrograms were administered into the ear artery in amounts of 1 ml./kilogram of body weight.

A total of 25 rabbits were used in this study divided into 5 equal groups. However, one rabbit died from technique problems during preliminary studies. The groups were treated as follows:

Group I Control group, no injections. 4 rabbits.

Group II Inoculated with benzene and olive oil until the leucocyte count was depressed to 1500 WBC ± 500 cells, this varied from 5 to 13 days. At this point no further injections were made and the animals allowed to recover on their own. Samples of blood were taken from the marginal ear vein to monitor the effects of benzene and recovery on each animal. WBC and RBC were counted using standard Coulter counter procedure. Hematocrits were done and two slide smears were made and stained with Wright's stain for differential counts. 5 rabbits.

Group III Leucogenenol was injected into the ear artery in the amount of 2.0 µg./ml./Kg of body weight. Samples of blood were taken every other day and just before the injections of leucogenenol. The blood samples were used

to determine the WBC and RBC counts, hematocrits, and differential counts. 5 rabbits.

Group IV Inoculated with benzene and olive oil until the WBC counts had reached 1500 ± 500 cells, this varied from 5 to 13 days. The benzene injections were stopped and 0.2 µg/ml/kg of leucogenenol given every other day for two weeks. Blood samples were taken from the marginal ear vein to monitor the effects of both benzene and leucogenenol. The blood samples were used to determine WBC and RBC counts, hematocrit, and differential counts. 5 rabbits.

Group V Inoculated with benzene and olive oil until the WBC counts had reached 1500 ± 500 cells. The benzene injections were stopped and 2.0 µg/ml/kg of leucogenenol given every other day for two weeks. Blood samples were taken from the marginal ear vein to monitor the effects of both benzene and leucogenenol. The blood samples were used to determine WBC and RBC counts, hematocrit, and differential counts. 5 rabbits.

The animals were observed several times daily. Injections of benzene and olive oil were administered subcutaneously above the rear leg. Injections of leucogenenol

were administered into the ear artery. During injections and bleedings, the rabbits were restrained either in a box designed for that purpose, or by an assistant. The assistant proved to be the better choice, since keeping the animal calm was of great importance during injections of benzene and olive oil.

Peripheral blood sampling was accomplished by rinsing one ear with xylene, which was used as a rubificant, and nicking the marginal vein with a sterile 20 - 25 gauge needle, and allowing the blood to pool. Approximately 20 microliters of blood was drawn with a micropippette. Blood smears, which would later be stained with Wright's reagent for differential counts, were immediately prepared. Solutions for the Coulter counter were prepared from the blood sample. On several occasions, 1 ml. of blood was drawn from the ear artery to be used in making: WBC and RBC counts, hematocrits, differential counts, hemagglutination, and complement-fixation tests. The blood drawn for these tests was obtained by using a sterile, heparinized 1 ml. disposable syringe. The blood was then evacuated into a 2 ml. vacuumized tube with 0.05 ml. of 7.5% EDTA solution to prevent clotting. Serum was later removed from these samples following centrifugation. The serum was placed in Durham tubes and frozen for later antibody studies.

Sheep red blood cells were injected into each animal as soon as the number of WBC's approached 1500 ± 500 cells,

this varied with each animal, but usually took from 5 to 13 days. The SRBC's were given again within 4-5 days of the original injection. Serum was removed from these animals within 4-5 days of the last injection of SRBC's.

RESULTS

The effects of leucogenenol stimulation of neutrophil and lymphocyte peripheral blood levels in benzene-induced leucopenic rabbits were measured by white blood cell counts, differential counts, and hemagglutination and complement-fixation titers. Red blood cell counts were obtained, along with hematocrits, to monitor the effects of benzene on these cells. The results of the red blood cell counts are not shown here because they are not pertinent to the outcome of this study.

Figures 1-5 show results obtained from white blood cell counts using standard Coulter Counter techniques.

In Figure 1, the controls, there can be seen extreme variations in the white cell counts of rabbits whose only trauma was having their ears nicked and injected with sheep red blood cells. These animals were sampled every other day, over a period of fourteen days. It should be noted that the fluctuations occurred early in the study and that with repeated handling the white cell counts gradually dropped to a stationary level.

Figure 2 illustrates the white cell counts of rabbits

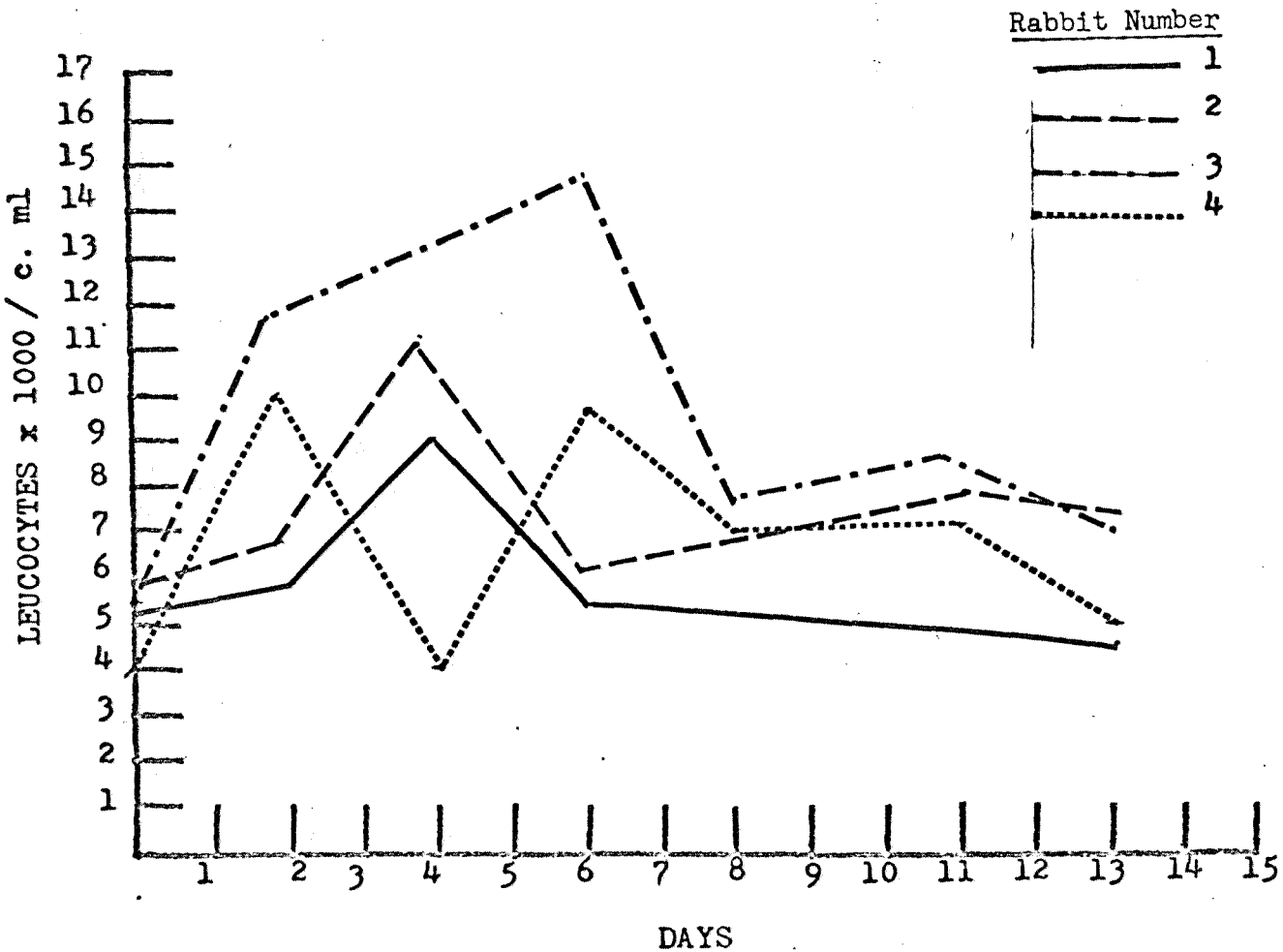

Figure 1. Control rabbits, no injections except for sheep red blood cells to test for antibody titers

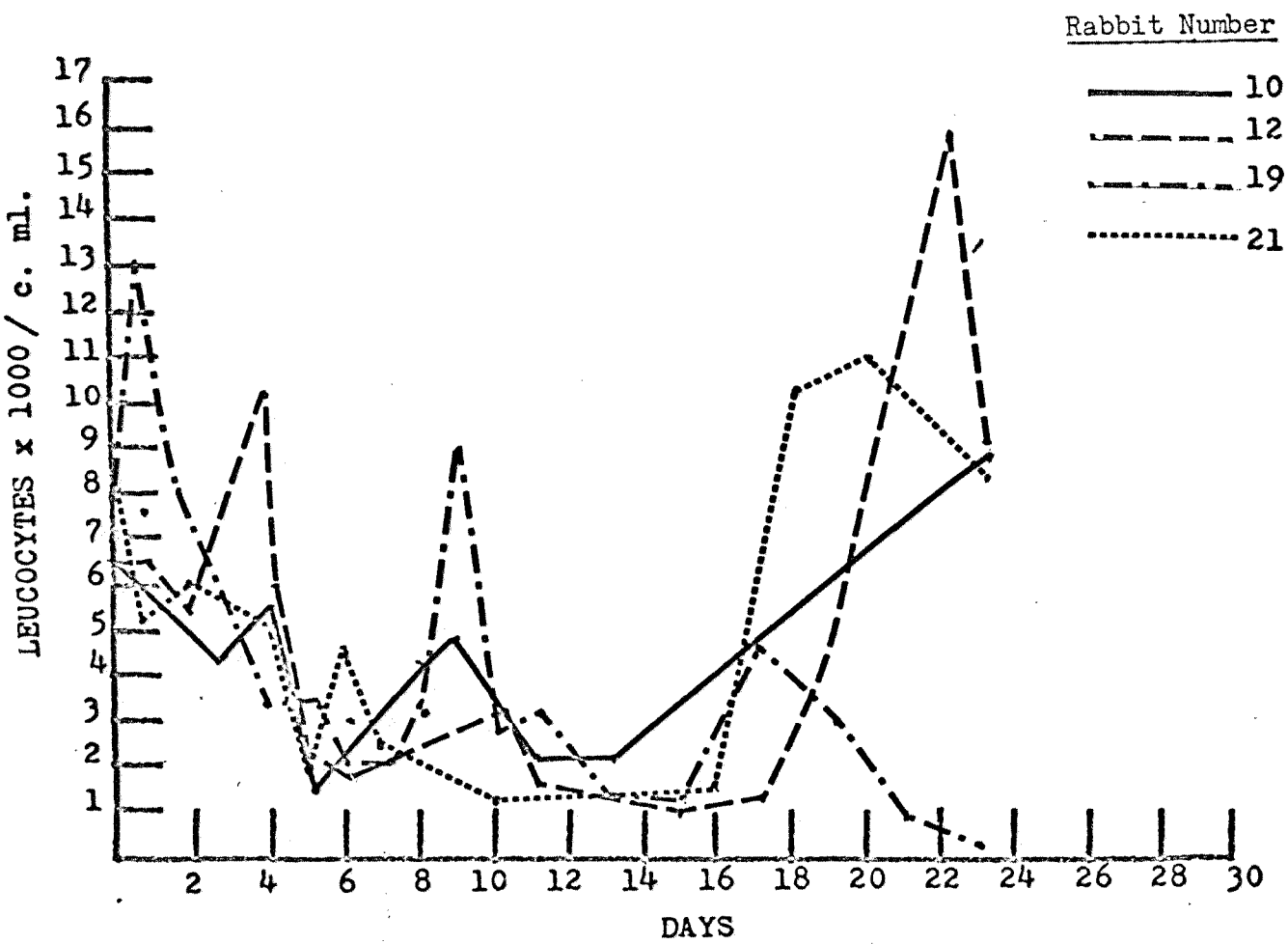

Figure 2. Rabbits injected with benzene and olive oil, to depress the leucocytes and then injected with 2.0 ug. of leucogenenol

injected with benzene and 2.0 µg of leucogenenol. The data indicates that leucogenenol initiated an increase in the white cell counts and that this increase was sustained over a period of time. The white blood cell count of rabbits treated with benzene and olive oil alone is shown in Figure 3. The effects of benzene on the peripheral white blood cells is quite dramatic. Fifty percent of those animals so treated, succumbed to a secondary infection and died on the twelfth day of the experiment. The white blood cell levels of those rabbits that did survive the study increased to a high level and then dropped to almost the same level as when initially sampled.

Figure 4 illustrates the effects of benzene and olive oil in rabbits followed by an injection of 0.2 µg leucogenenol. The effects of benzene were the same as those shown in Figure 3. The rabbits in this group showed very individualistic reactions to the benzene. After the injection of leucogenenol there was an increase in the white cell counts. Although the increase was apparent, it was not sustained and towards the end of the experiment there was a gradual decline in the white cell counts. All but one animal survived the experiment.

Figure 5 shows similar individual reactions to benzene and olive oil, as did the animals in group four. The administration of 2.0 µg of leucogenenol did have a more pronounced effect on the white cell levels of these animals.

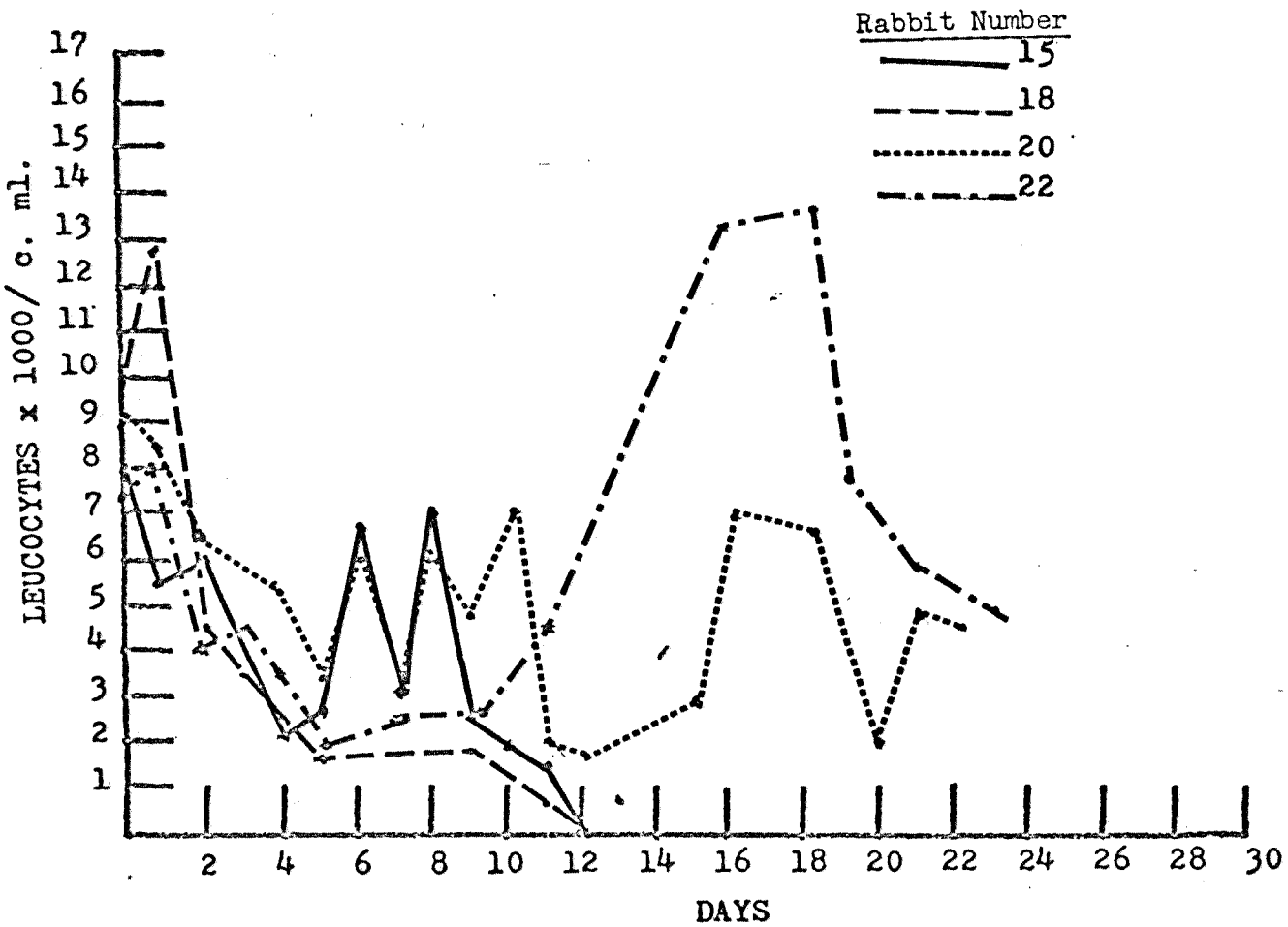

Figure 3. Rabbits injected with benzene and olive oil to depress the leucocytes and then allowed to recover with no further injections

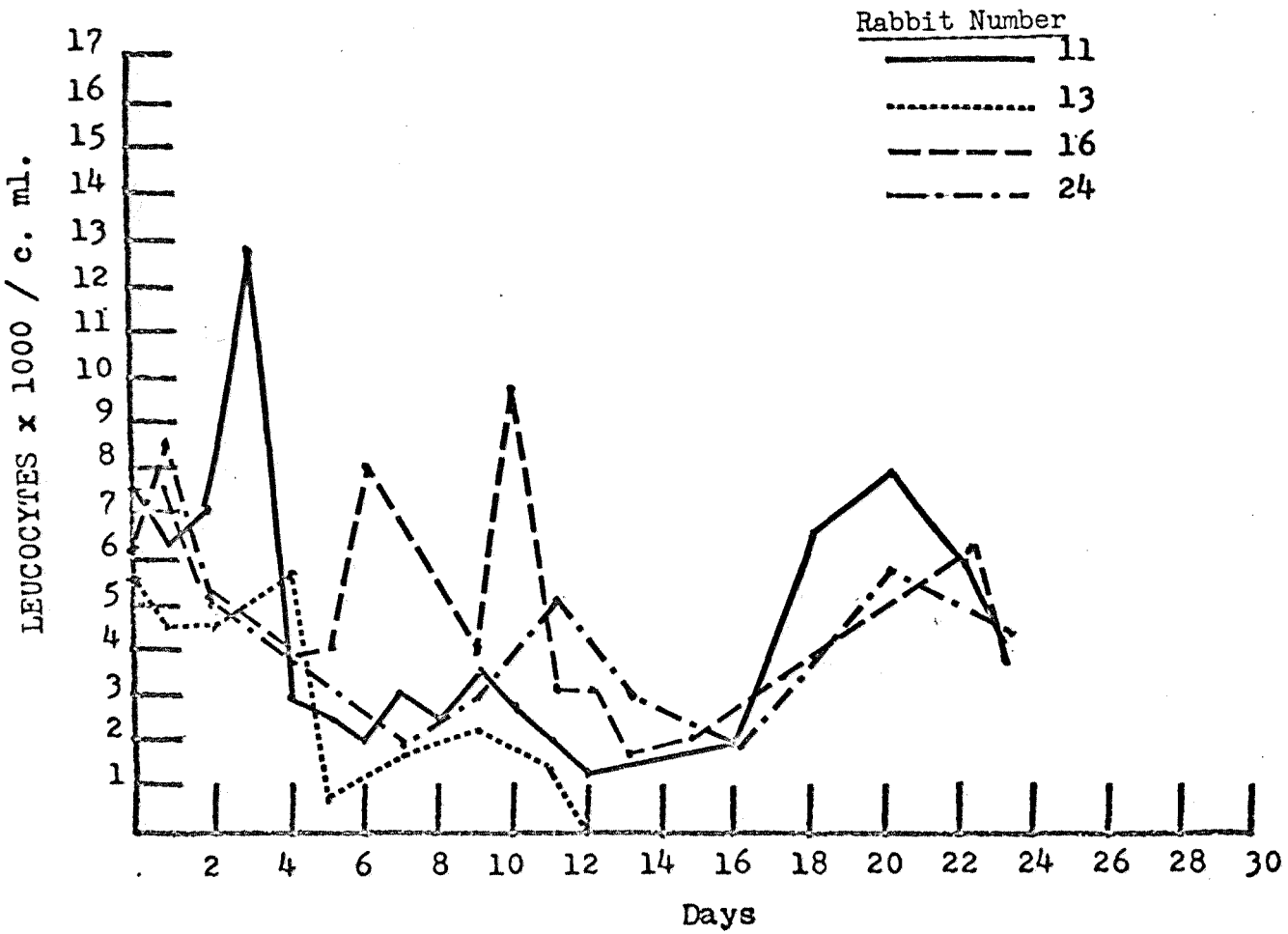

Figure 4. Rabbits injected with benzene and olive oil to depress the leucocytes, and then injected with 0.2 ug. of leucogenenol

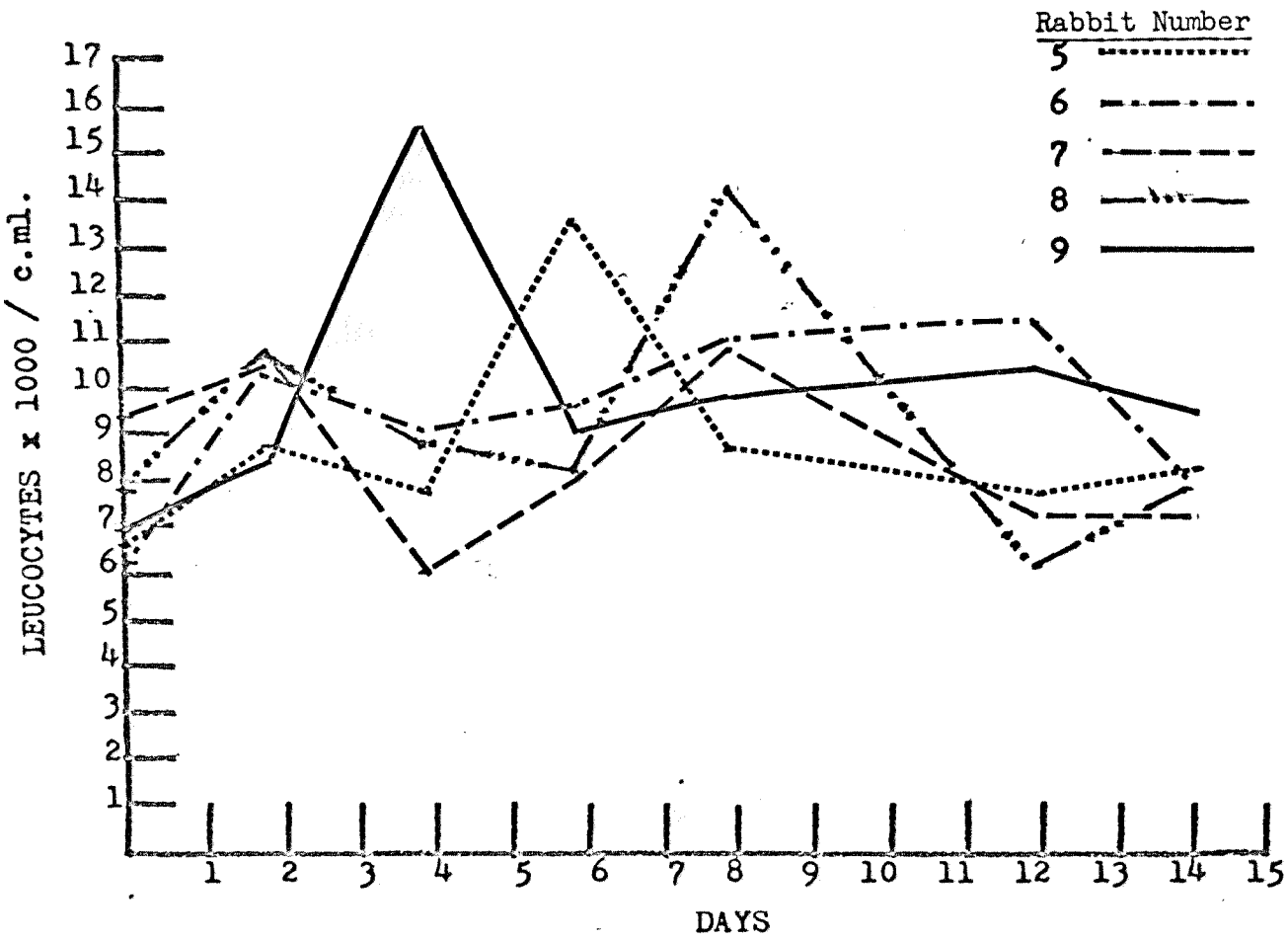

Figure 5. Rabbits injected with 2.0 ug. of leucogenenol

The increase went far above the initial white blood count, and did not fall below this level at the end of the experiment. One rabbit did not survive the experiment, but even in this animal there was an increase in white blood cells to almost 4500 cell/cubic millimeter and then a gradual but fatal decline.

Tables 1 and 2 show results obtained from hemagglutination and complement-fixation tests using standard microtiter techniques.

Table 1 shows the comparison of the no treatment controls and the leucogenenol 2.0 μg groups. The first column, pre-SRBC's, shows the amount of antibodies present at the onset of the experiment. Both the hemagglutination and complement-fixation titers are low enough to be considered insignificant. The second column, post-SRBC's I, shows titers of blood samples taken after the sensitizing doses of sheep red blood cells were injected into the rabbits. The mean titers of these two groups indicates that leucogenenol induced a two-fold response in terms of hemagglutination and a ten-fold response in terms of the complement-fixation. The third column, post-SRBC's II, the leucogenenol maintained an almost two-fold response in the hemagglutination titers. The complement-fixation titers were very high and may have been higher, but a second plate was not prepared.

Table 2 compares the hemagglutination and

Table 1. Hemagglutination and Complement-Fixation Titers of Rabbits Given No Injections, and Rabbits Given Injections of Leucogenenol 2.0 µg.

Group	Rabbit	Pre-SRBC's Titers HA	Pre-SRBC's Titers C'Fix.	Post-SRBC's Titers I HA	Post-SRBC's Titers I C'Fix.	Post-SRBC's Titers II HA	Post-SRBC's Titers II C'Fix.
Control	1	neg.	neg.	neg.	neg.	128	2048
	2	neg.	4	neg.	4	512	2048
	3	2	8	2	8	256	2048
	4	2	4	2	4	256	2048
Mean Titer		1	4	1	4	288	2048
Leuco. 2.0 µg.	5	neg.	2	neg.	2	256	2048
	6	4	64	4	64	512	2048
	7	4	64	4	64	256	2048
	8	4	64	4	64	512	2048
	9	2	2	2	2	512	2048
Mean Titer		2.8	39	2.8	39	409	2048

Table 2. Hemagglutination and Complement-Fixation Titers of Rabbits Injected with Benzene Only; Benzene and Leucogenenol 0.2 µg.; and Benzene and Leucogenenol 2.0 µg.

Group	Rabbit	Pre-SRBC's Titers HA	Pre-SRBC's Titers C'Fix.	Post-SRBC's Titers I HA	Post-SRBC's Titers I C'Fix.	Post-SRBC's Titers II HA	Post-SRBC's Titers II C'Fix.
Benzene Only	15	2	2	-	-	8	64
	18	2	2	2	2	8	512
	20	neg.	2	64	64	32	256
	22	4	4	2	2	32	64
Mean Titer		4	5	17	17	20	224
Benzene and Leuco. 0.2	11	2	2	2	4	16	256
	13	4	2	2	4	16	256
	16	4	4	16	4	16	256
	24	2	2	8	32	64	1024
Mean Titer		3	3	7	11	28	448
Benzene and Leuco. 2.0	10	neg.	neg.	4	2	64	512
	12	2	4	16	4	neg.	neg.
	19	4	4	128	2	32	128
	21	neg.	neg.	2	2	8	64
Mean Titer		1.5	2	37.5	2.5	26	176

complement-fixation titers of animals injected first with benzene and then either allowed to recover on their own or injected with leucogenenol to determine if the substance would stimulate a faster recovery of the immune system. Leucogenenol has been described by Rice (1971) as being capable of increasing the antibody titers. This conclusion cannot be supported by the data presented here. The most significant difference occurred between the hemagglutination titers of the benzene only group, and the two groups injected with leucogenenol. By comparing these three groups with the control group, it can be seen that benzene severely retards or damages the immune response. Leucogenenol in doses of either 2.0 μg or 0.2 μg does not increase the recovery of a system damaged by benzene.

The hemagglutination titers of the benzene only group showed a similar recovery response as did those of the leucogenenol groups. The complement-fixation titers showed a two-fold increase in those animals injected with 0.2 μg leucogenenol as compared to animals injected with 2.0 μg leucogenenol or the animals left to recover on their own.

The overall view of Figure 6 indicates three major points in the study. In group A, the blood samples were taken and smears were made before the rabbits were given any injections. This is a reference point to which the other columns are compared. Group B shows the second point

Group A: Smears taken before any injections

B: (*) Sample taken at the middle of the experiment
(**) Indicates the point at which Benzene injections were stopped.
1500 white blood cells ± 500 cells

C: Smears taken at the end of the experiment

1 Untreated controls

2 Benzene only

3 Leucogenenol 2.0 µg only

4 Benzene and Leucogenenol 0.2 µg

5 Benzene and Leucogenenol 2.0 µg

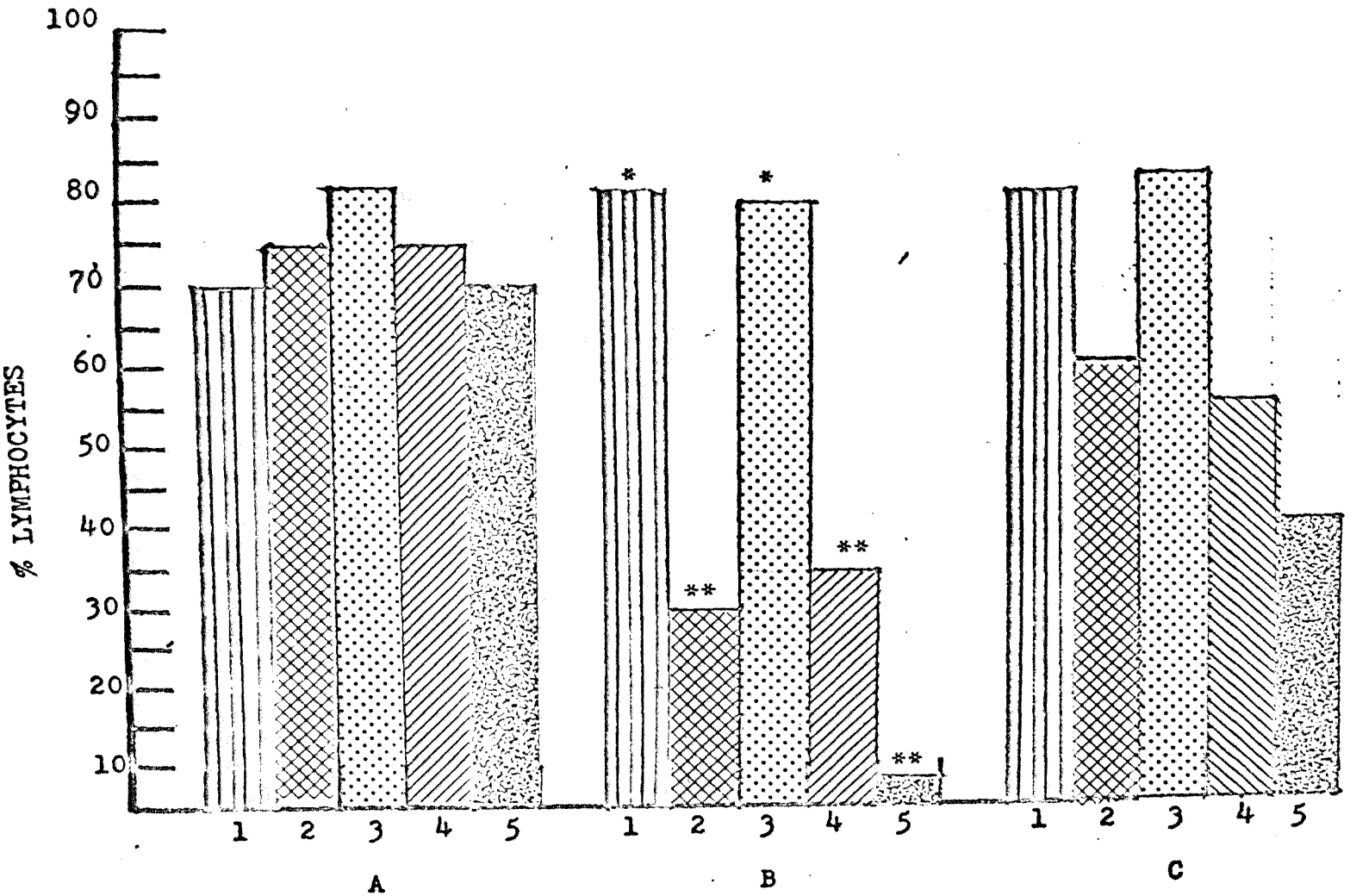

Figure 6. Average percentage of lymphocytes in the peripheral blood smears of rabbits.

to which this study was directed, namely a decrease in the white blood cell count of those rabbits injected with benzene and olive oil. Since the control group and the leucogenenol 2.0 µg group were not subjected to benzene, their numbers were obtained from the middle of the study, which was approximately the same time the other samples were obtained. The percentages indicated for those animals injected with benzene are not accurate percentages, because in many instances the counts were extremely low. In all instances when 100 cells were not available on a slide, at least ten passes over the slide with the oil immersion lens was made. Group C indicates the percentage of lymphocytes found in those animals surviving the study.

DISCUSSION

Leucogenenol was found to be a stimulator of leucocytosis and antibody production. Injection of leucogenenol into healthy rabbits seemed to have little or no effect on their immune systems. Rice and Ciavarra (1971) found similar results when they compared the antibody titers of splenectomized rats and normal rats. The splenectomized rats showed a marked increase, while the leucogenenol had no effect on the normal rat antibody titers. Similar results were obtained by Rice, Lepick, and Hepner (1970) who found that leucogenenol stimulated the antibody titers of irradiated mice and they found no significant change in normal

mice. The above authors used mice or rats in their experiments, while in this study rabbits were used producing similar results. The no treatment controls and the leucogenenol only group both had high antibody titers, illustrating that leucogenenol has little effect on normal rabbits. In some instances the leucogenenol showed higher antibody titers than the no treatment controls, but this wa not considered significant. In the three experimental grou whose immune systems were suppressed by injections of benzene, the group with injections of 0.2 µg of leucogenenol showed a higher complement-fixation titer at the end of the experiment than did the other experimental groups.

Rice, Connolly, Aziz, and McCurdy (1971) stated that leucogenenol causes a release of large numbers of neutrophils and stimulates the rate of lymphocyte formation from precursor cells. This conclusion is not confirmed in this study with leucogenenol. Although it is not confirmed it is not contradicted because of the differences in the animals used and the use of benzene to intoxicate the immune system. The data in this study indicates that an animal, the rabbit, intoxicated with benzene will have a larger number of lymphocytes, if it survives, than a similar animal treated with leucogenenol. The "if it survives" is an important phrase, because leucogenenol seems to be responsible for the survival of almost 90% of the benzene treated rabbits, even though the lymphocyte percentages of those that survived

the benzene only treatment were higher than those that survived the leucogenenol treatment.

In reading the blood smears which were made over the course of the study, a small nucleated cell, about the size of an erythrocyte, began to become evident in ever increasing numbers as the experiment continued. At first these cells appeared to have some significance and seemed to agree with the work of Rice, Connolly, Aziz, and McCurdy (1971). However, further analysis showed these cells to be present in the benzene only group, and in numbers similar to those groups injected with leucogenenol. Since the benzene only group did not receive any leucogenenol, then the presence of these cells must be a result of a mechanism other than leucogenenol stimulation.

The rabbit was selected for this study because of the large amounts of blood sampling necessary to monitor the effects of benzene on the peripheral leucocytes. However, in the course of reviewing the literature, Rice (1968) found that leucocytosis of rabbits varied so much as to give erroneous conclusions. One author, Schermer (1967), stated that one need only look askance at a rabbit to produce a change in its blood picture. If further studies are done, animals other than the rabbit should be used as the experimental subject.

Benzene is a most interesting substance both in terms of its widespread use in home products and in industry. Its

effects on the hemopoietic system are equally fascinating and equally variable. The effects of leucogenenol in repairing the hemopoietic system damaged by benzene, has been shown, in this study to be somewhat successful.

The effects of leucogenenol on the leucocyte level of the blood, and the effect of small amounts suggests that leucogenenol may be hormonal. It may be part of the mechanism which maintains the leucocyte level in a normal human. Hypersecretion may bring about elevated leucocyte levels in time of stress or infection and an extreme hypersecretion may produce a leukemic condition. The presence of a feedback mechanism or an antagonistic hormone would be a subject for further study.

SUMMARY

This study was designed to investigate the effects of leucogenenol on leucocytosis in rabbits. By depressing the leucocyte level with benzene, the extent of leucocytosis by leucogenenol could be observed. Although leucogenenol was effective in bringing about an increase in leucocytosis in animals intoxicated by benzene, there is evidence that leucogenenol was ineffective in changing the blood picture characteristic of normal animals.

The control group of animals were observed to have large fluctuations in their leucocyte counts, indicating wide variations in the blood profiles of rabbits.

In test animals injected with 2.0 µg of leucogenenol, the number of white blood cells increased to a level comparable to the controls, indicating the ineffectiveness of leucogenenol activity on normal blood marrow tissue.

In the group of animals injected with benzene only and allowed to recover on their own, two rabbits died within 2 to 6 days after having their leucocytes depressed below 1500 cells per cubic millimeter.

In the group of animals in which the leucocyte level was depressed by benzene and then injected with 0.2 µg of leucogenenol, only one of the four animals died after receiving the leucogenenol treatments. The blood profiles of the surviving rabbits did not show as great an increase in the number of white blood cells as did those with no leucogenenol.

The group of rabbits receiving 2.0 µg of leucogenenol after the depression of white blood cells by injections of benzene, showed a larger number of white blood cells formed during the recovery period. All animals in this group survived the experiment.

Peripheral blood smears and Coulter counts of the white blood cells of all the benzene intoxicated animals were influenced by the presence of a nucleated embryonic cell which was determined to be a reticulocyte. The presence of this cell may be due to the recovery response of the hemopoietic system against the insult of benzene, and not to

the effects of leucogenenol. The animals injected with leucogenenol only did not exhibit these embryonic cells.

Leucogenenol was also shown unable to repair the immune system damaged by benzene intoxications.

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